Original Article

Estrogen Accelerates Wound Angiogenesis in Rats

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ABSTRACT

Background and Objective: **Angiogenesis is a complex program of several steps and it is tightly regulated by pro- and anti-angiogenic factors. Angiogenesis is one of the key elements in cutaneous wound healing and skin cancers. Estrogen seems to have positive modulating effect on cutaneous wound healing and this effect may be explained by its angiogenic properties. This study aims to investigate the effect of estrogen on cutaneous wound angiogenesis in rats through histological criteria.**

Materials and Methods: **This was an experimental study which was carried out at Esfahan University of Medical Sciences in August 2007. Forty rats were randomly allocated into two groups and an experimental wound was induced in their skin. Wounds in the case group were treated with daily topical estrogen and gentamicin, while the controls received only topical gentamicin. After 14 days of treatment, biopsies were obtained.**

Results: **Evaluation of wounds through a validated histological scoring system revealed significant difference between control and treated mice. The latter exhibited increased microvasculature and significantly higher scores of angiogenesis.**

Conclusion: **Our study suggests that topical estrogen is able to increase cutaneous wound angiogenesis considering objective histological criteria.**

Key words: **Angiogenesis Inducing Agents, Wound healing, Estrogen.**

Introduction

Angiogenesis, new vessel formation, is a pivotal phenomenon in many physiological and pathologic conditions. It plays a crucial role in wound healing, tumor progression and in perpetuation of chronic inflammatory diseases (1). Angiogenesis is a complex phenomenon, a program of several steps and it is tightly regulated by pro- and anti-angiogenic factors (2-4). The steps include activation of endothelial cells (ECs) by angiogenic mediators resulting in secretion of EC proteases (4-6), degradation of previously existing basement membrane and extracellular matrix (ECM), attachment and migration of ECs to stromal matrix, EC proliferation, formation of primary capillary sprouts, alignment, lumen formation within the sprout and differentiation into tubular structures, and establishment of a new basement membrane (7,8). Angiogenesis is up-regulated due to hypoxic

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conditions (e.g., in continuous accumulation of cancer cells), or constitutive activation of genes producing growth factors with angiogenic activities, i.e., vascular endothelial growth factor (VEGF). The recruitment of fibroblasts and inflammatory cells may also contribute to the angiogenesis process (9,10).

Topical estrogen administration results in significant progress of cutaneous wound healing (11,12). It can increase the extent of wound healing by reducing wound size and stimulating matrix deposition (13-17). The effect of estrogen on wound healing can be explained by its angiogenic properties. Estradiol enhances fibroblast growth factor (FGF)-2-induced angiogenesis in an in vivo model (18). In addition, other investigators have shown that disruption of the estrogen receptor gene reduces FGF-2-induced angiogenesis (19). A role for estradiol in modulating angiogenesis is suggested by the fact that neovascularization physiologically occurs in the uterus during the menstrual cycle (3,20). In avian osteoclast precursors, estrogens have been demonstrated to enhance αvβ3 integrin expression by stabilizing its mRNA (3,21). Studies show that αvβ3 is involved in EC migration, angiogenesis, and are required for the survival and maturation of new blood vessels (22,23).

Estrogen stimulates endothelial cell adhesion to ECM proteins, protease production and migration through Boyden chambers (3,24). Estrogen-promoted endothelial cell migration is relevant for angiogenesis and may also contribute to repair an injured endothelium. Estradiol also promotes late steps in angiogenesis such as differentiation into tubular structures (3).

Therefore, in this study we tried to investigate the effect of estrogen on cutaneous wound angiogenesis through histological criteria.

Materials and Methods

This was an experimental study which was carried out in animal lab of Isfahan University of Medical Sciences in August 2006. All animal procedures were in accordance with the declaration of Helsinki and the Guide for the Care and Use of Laboratory Animals. We performed our study on a total of 40 albino male rats (a body weight of 150 ± 5 grams and an age of 3 ± 0.5 months). They were randomly allocated into two groups. Following a 0.5 ml intradermal injection of 1% xylocain solution on the right flank, we induced a circle-shaped wound of 2 cm in diameter in anesthetized skin so that the fascia was exposed. The skin specimens were sent for pathologic study. The wounds in case rats were treated with a daily topical dose of 0.5 mg estrogen and gentamicin 0.1%, while the control group rats received only topical gentamicin 0.1%. During the course of experiment which lasted for 14 days, the animals were housed one per cage, maintained under controlled environmental conditions, (12-hour light/dark cycle and a temperature of 20-25 ˚C) and provided with standard laboratory food and water. The wounds were appropriately covered with moist saline dressing to prevent rats from grooming or licking.

After 14 days of treatment, the animals were again anesthetized and a 5 mm biopsy was obtained from each wound. This biopsy included skin, healed wound tissue and normal margin. The specimens were immediately fixed in 10% neutral buffered formalin overnight at room temperature. Subsequently, perpendicular sections to the antero-posterior axis of the wound area were washed in tap water, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin according to routine procedures. Five-micrometer thick sections of paraffin-embedded tissues were mounted on glass slides, hydrated in distilled water, and stained with hematoxylin and eosin. Slides were all filed and stored for comparison and scoring. Slides were scanned at ×10-20 magnification to briefly obtain and record an overview of morphologic features. A histological scoring system which was previously validated through similar experimental models (11) was used to record the degree of angiogenesis (Table 1). A systematic examination of random fields at an objective lens magnification of ×40 was used. At this level, histological scores were made from 20 random fields per section from each specimen and expressed according to literature (11, 25). We used an information recording sheet for each rat. The following histological variables were measured and scored: number of capillary lumens per site, presence of edema, congestion, fibrin deposition and hemorrhage.

Data were analyzed through independent student t-test, using SPSS version 10 and a statistical p value less than 0.05 was considered significant.

Results

Histological evaluation of wounds and the statistical analysis of data revealed significant difference between control and treated rats (Table 2). The former

group showed few newly formed capillaries (two to four per site) with evidence of hemorrhage, interstitial edema, fibrin deposition and generalized vascular congestion within the granulation tissue (Figure 1). In contrast, wounds in treated rats exhibited numerous capillaries (around 50 per site) within the granulation tissue, slight congestion and minimal interstitial edema but no evidence of hemorrhage, fibrin deposition or thrombosis which characterize the increase of microvasculature (Figure 2). According to the histological scoring system indicated in Table 1, 70% of estrogen treated rats indicated the criteria of score four of angiogenesis, while none of the rats in the control group expressed those feature ($p<0.05$). None of the rats in the case group were categorized in scores 1-2, while 10% and 60% of rats in the control group fit these categories respectively ($p<0.05$) (Table 2). Data presented in Table 2 significantly show that estrogen can enhance the histological process of angiogenesis in experimental wounds.

Table 1. Scoring of histological criteria for rate of angiogenesis

Score	Criteria of angiogenesis
0	Absence of angiogenesis, presence of congestion, hemorrhage, and edema
1	1-2 vessels per site, edema, hemorrhage, and congestion
2	3-4 vessels per site, moderate edema and congestion
3	5-6 vessels per site, slight edema and congestion
4	More than 7 vessels per site vertically disposed toward the epithelial surface

Table 2. Comparison of angiogenesis scores between control and estrogen treated groups

Groups / Score	Control group		Estrogen treated group		P -Value
	Frequency	Percentage	Frequency	Percentage	
0	2	10%	0	0%	<0. 05
1	12	60%	0	0%	<0. 05
2	5	25%	2	10%	<0. 05
3	1	5%	4	20%	<0. 05
4	0	0%	14	70%	<0. 05

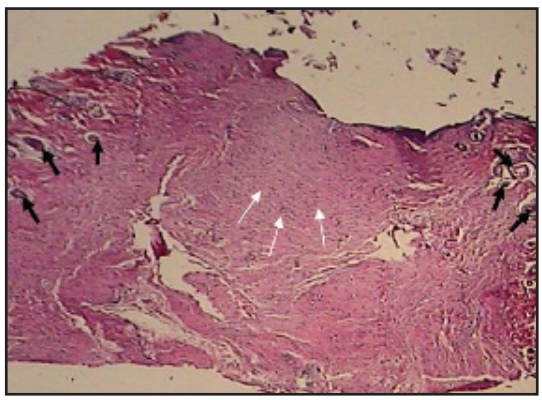

Figure 1. High power view light micrograph of control group wound with evidence of immature collagenous tissue, vascular congestion, very low capillary count (less than two to four per site) and severe inflammation. There is also absence of epithelialization and substitution of fibrous tissue (white arrows) for normal epithelium and hair follicles (black arrow) in both sides of wound (Hematoxylin and eosin; x20)

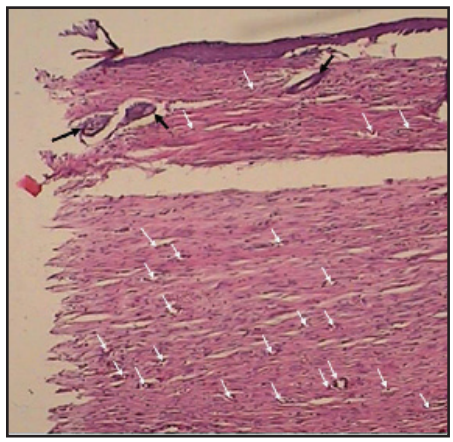

Figure 2. High power view light micrograph of estrogen treated wounds characterized by complete thick reepithelialization, well-formed granulation tissue and newly formed microvessels (white arrows) and hair follicles (black arrow) is observed (Hematoxylin and eosin; x20)

Discussion

Angiogenesis has a critical character in a number of physiological processes such as tissue repair, reproduction and development, as well as in disease states including inflammation and malignancies (4). Formation of new vessels has a compensatory role in diseases with vascular occlusion such as coronary artery disease, atherosclerotic limb ischemia, or vasculitis (3). Angiogenesis occurs continuously during growth and development, but the endothelium is quiescent during adulthood. There are two major exceptions to this rule: first, during injuries, wounds and chronic ischemia, and second, the physiological vascularization that occurs in decidual tissue during the menstrual cycle and throughout pregnancy (7). Angiogenesis occurs in consecutive phases. ECs play significant roles in all these phases. Angiogenic mediators may act directly on EC proliferation and migration or may indirectly stimulate the production of other angiogenic factors. Among the mediators, growth factors (including bFGF and VEGF) are the most potent agents (4-6). Previous studies recognized estrogen as an angiogenic mediator (18, 25); these studies suggest that VEGF and bFGF linked to estrogen probably play an important role in neovascularization, as in placental development and growth (25). Estrogen stimulates bFGF mRNA expression (4,7) and so it recovers bFGF expression to activate angiogenesis in castrated animals with down regulated angiogenesis. It also enhances endothelial cell activity which is important in neovascularization, suggesting a promoting influence of estrogen on angiogenesis (25).

Obviously, estrogen increases expression of a number of genes in endothelial cells including endothelial NO synthase and therefore estrogen enhances nitric oxide production and release by endothelial cells (7,25). Estrogen has also antioxidant properties and prevents nitric oxide degradation, increasing; consequently, nitric oxide availability (3). Nitric oxide possesses several biological roles in the process of cutaneous wound healing including VEGF-induced neoangiogenesis (3). Recent studies reveal that topical estrogen leads to significant progress of cutaneous wound healing (11). It stimulates matrix deposition and so diminishes the wound size (24). Topical estrogen ameliorates all histological healing criteria in acute incisional wounds in male rats (11). A study by Ashcroft et al demonstrates that topical estrogen can reverse the marked delay in repair of experimental wounds presented by ovariectomized young female rats; this shows that estrogen modulates both the rate and quality of wound healing (15).

As mentioned before, angiogenesis is one of the key processes in cutaneous wound healing (11). In the healing process, angiogenic capillary sprouts invade the wound clot and soon form a microvascular network throughout the granulation tissue. Studies on this issue show that as collagen accumulates in the granulation tissue to produce scar, the density of blood vessels diminishes. A dynamic interaction occurs among endothelial cells, angiogenic cytokines, such as FGF, VEGF, TGF- β, angiopoietin, mast cell tryptase, and ECM environment (2,12).

All this experimental evidence promoted us to study the effect of topical estrogen on cutaneous wound angiogenesis in experimental wounds. Our results suggest that topical estrogen is able to improve angiogenesis in experimental wounds in rats. This improvement can be assessed through a qualitative and quantitative objective scoring system. Further studies are suggested to determine the specific angiogenic factors, mediating the effect of estrogen on cutaneous wounds.

Conclusion

Our study suggests that topical estrogen is able to increase cutaneous wound angiogenesis considering objective histological criteria.

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